Cultivation of oyster mushrooms on cassava waste

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*Cassava is a major food crop for approximately 700 million people, especially in African countries. A large quantity of waste is produced during processing of cassava, mainly consisting of tuber peels. Although previous research has shown that these peels can be an ingredient for substrate to cultivate mushrooms, yields were usually inferior compared to traditional substrates such as sawdust. In a project funded by the European Union (www.fp7-gratitude.eu/) trials were done for the production of oyster mushrooms using fermented peels and stems from cassava crops produced in Ghana. Four mushroom strains representing two species (*Pleurotus ostreatus *and* Pleurotus pulmonarius*) were grown on fermented substrates made from cassava waste (peels and stems) without further heating/sterilization. Peels and cassava stems were tested in different ratios and supplemented with different amounts of rice or wheat bran. All substrates colonized quickly (15–16 days) and time to pinning varied between 18 and 24 days. The* P. pulmonarius *strains produced three flushes within 47 days (starting from inoculation) and the* P. ostreatus *strains needed 57–63 days completing flush 3. Biological efficiencies after three flushes varied between 38 per cent and 100 per cent. Addition of bran (rice or wheat) increases yields significantly. The trials have shown that cassava waste can be used well for the production of oyster mushrooms and that production from substrates containing up to 75 per cent cassava peels compares well to yields obtained on the traditional sawdust-based substrates.*

Keywords: *Pleurotus* spp., cassava waste, biological efficiency, fermentation

DURING THE PRODUCTION OF CASSAVA, large quantities of peels and, to a lesser extent, plant stems are produced. At present, only part of this waste is used, mainly as feed for goats. In many places where cassava is cultivated and processed, heaps of cassava peels are discarded along the roads especially in wet seasons and can cause unpleasant odours and unhygienic conditions. A valorization of cassava waste would not only solve this environmental problem but can also contribute to the local economy. Previous reports on the utilization of cassava waste for the cultivation of grey oyster (*Pleurotus ostreatus*) and lung oyster (*Pleurotus pulmonarius*) mushrooms have

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shown that yields were reasonable but still lower than those obtained on traditional substrate prepared from sawdust (Adebayo et al., 2009; Onuoha et al., 2009; Obodai et al., 2014). The objective of this research was, therefore, to improve cultivation of oyster mushrooms using cassava waste to such an extent that it can compete well with cultivation on traditional substrates. One of the bottlenecks in using cassava waste is the low nitrogen content of especially the peels of cassava (Burns et al., 2012), one of the ingredients known to enhance the productivity of mushrooms (Naraian et al., 2009). In this paper trial results are presented in which substrate was prepared from cassava waste (peels and stems) supplemented with rice or wheat bran in order to enhance the nitrogen content of the substrate mixtures. Wheat and rice bran are known for their high protein content (Fabian & Ju, 2011). Cultivation of both oyster mushroom species for three flushes showed that the addition of these bran supplements increases yields considerably, up to levels comparable or even better than on traditionally used sawdust-based substrates. In addition, cassava waste-based substrates are colonized in a shorter time than sawdust and thus allow more crop cycles per year, increasing the productivity even more. The cassava peels and stems were fermented for six days and inoculated subsequently with spawn without sterilization of the substrate. Infections that are sometimes seen in oyster mushroom crops (green mould) were not observed by eye indicating that, when done properly, cultivation on fermented cassava waste can be cost-effective since energy required for sterilization or high temperature pasteurization is costly.

Materials and methods

Substrate

Fermentation of the substrates and cultivation of mushrooms were done at Unifarm (experimental farming system of Wageningen University and Research Centre). Cassava peels and stems were obtained via the Food Research Institute, Accra, Ghana and originate from a regular cassava production site in Ghana. Peels (sun-dried in Ghana) and stems were packed in nylon sacks and submerged in water for three days and subsequently drained for one day to remove excessive water. Mixtures of peels and stems were then prepared. Chalk and bran were subsequently added and mixtures were thoroughly homogenized. For each type of substrate mixture (Table 1) three trays were filled, each containing *c*. 27.8 kg of substrate. Trays were put in a mushroom cultivation room of Unifarm, Wageningen UR, and the room was heated by steam up to 45°C. This was done to mimic fermentation in large heaps where preheating to start fermentation is not needed. Trays were fermented for 6 days. During fermentation temperature was measured and most substrates reached temperatures of at least 65°C for 24 hours. After cooling to 22°C, each substrate type was mixed with spawn (60 ml, i.e. 30 g of spawn/kg) and distributed over bags (2 kg inoculated substrate per bag). Spawn was prepared as described by Tuyen et al. (2012). Bags were obtained from Microsac (www.saco2.com/) and closed with tape after filling. Spawn run (mycelial growth) was done in a growing room at 25°C for *c*. 15 days.

Table 1 Composition of cassava waste-based substrates

No.	Peels	Stems	Ratio (peels/stems)	$CaCO_3$	Rice bran	Wheat bran
	% (w/w)	% (w/w)	% (w/w)	% (w/w)	% (w/w)	% (w/w)
1	71.5	26.5	2.7:1	2	0	0
2	70.75	26.25	2.7:1	2	1	0
3	68.5	26.5	2.6:1	2	6	0
4	66	27	2.4:1	2	12	0
5	70.75	26.25	2.7:1	2	0	1
6	68.5	26.5	2.6:1	2	0	6
7	66	27	2.4:1	2	0	12
8	49	49	1.0:1	2	0	0
9	46.5	50.5	0.9:1	2	1	0
10	45.5	49.5	0.9:1	2	6	0
11	44.5	48.5	0.9:1	2	12	0
12	46.5	50.5	0.9:1	2	0	1
13	45.5	49.5	0.9:1	2	0	6
14	44.5	48.5	0.9:1	2	0	12

Mushroom varieties

Two of the varieties tested were obtained from the Federal Institute of Industrial Research, Oshodi (FIIRO): one *P. ostreatus* (MES14030) and one *P. pulmonarius* strain (MES14029). The other two strains were from the Plant Breeding collection of Wageningen UR: one commercial *P. ostreatus* (MES 03448) and one *P. pulmonarius* strain originating from Brazil (MES01997).

Cultivation

After colonization, four slits each approximately 8 cm long were made on each bag (one in all four sides) with a sharp knife to allow the formation of mushrooms. The room was subsequently vented with fresh air and cooled to 20°C with relative humidity of 88 per cent. Light was on for 12 hours/day.

Statistical analysis

Anova was carried using Genstat for Windows (17th edition; VSN International, Hemel Hempstead, UK).

Results and discussion

Fermentation of substrates

Fermentation of each type of substrate was done in trays each containing 27.8 kg of substrate. Degradation and spontaneous fermentation of starch and other easily degradable compounds in the peels by microorganisms will usually heat up the

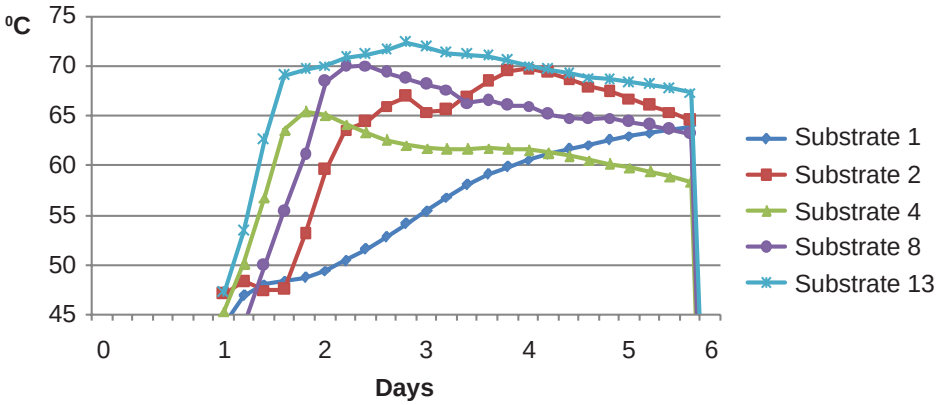

Figure 1 Temperature profiles of the different substrates during the 6 days of fermentation. The temperatures are depicted after the substrates have reached room temperature (45°C). The number in each curve refers to the substrate mixtures listed in Table 1. The irregularity in some curves (dip in temperature) is due to the occasional improper functioning of temperature probes

substrate. This results in killing insects and pathogenic organisms and making the substrate more selective for growth of white rot fungi. If this fermentation process runs properly, i.e. if considerably high temperatures are reached in a short time, it will eliminate the need for sterilization of substrate and it will lower the technical demands and costs for substrate preparation. The temperature during fermentation was, therefore, monitored for each substrate. Since only a relatively small amount of substrate is present in each tray, the heat will easily be lost to the environment. For that reason the environment (the room) was heated up to 45°C to mimic fermentation in large heaps where temperature is easily built up. All types of substrate reached the room temperature of 45–47°C within 1.5 days. After that, temperatures for different substrates rose in different ways but all increased considerably in temperature, indicating microbial activity. The substrates with wheat bran and a 50:50 ratio of cassava peels and stems reached the highest temperatures (69–73°C; Figure 1).

A visual inspection of the substrates after fermentation showed that most substrates were well colonized by thermophilic microorganisms (whitish appearance), especially trays with wheat bran which showed a dense colonization by thermophiles. The presence of thermophilic microorganisms is usually a good indication for a proper fermentation of the substrate (Antonella et al., 2005). The addition of wheat bran up to 12 per cent was considered too high since these were the only substrate mixtures that had an ammonia smell at the end of the fermentation period. That might indicate that in these substrates production of ammonia still continues due to the excess of nitrogen rich supplements. The weight loss of substrates during fermentation was on average 16 per cent (based on wet weight).

Production profile

Daily visual inspections showed that 15 days after spawning (inoculation), most of the substrates were colonized. Four slits were then made in the bags (one

Table 2 Average time needed to complete three flushes for strains

Strains	*Mean (days after inoculation)*
P. pulmonarius (commercial)	44.9[a]
P. pulmonarius (Nigeria)	46.9[a]
P. ostreatus (Brazil)	55.87[b]
P. ostreatus (Nigeria)	62.68[c]

Table 3 Statistical analysis on time needed to complete three flushes for the type of substrate (Tukey's 95% confidence intervals)

Substrate	*#Peels/Stems*	*Bran (%)*	*Mean (days after inoculation)*
11	1:1	12 RB	47.00[a]
4	3:1	12RB	47.48[a]
3	3:1	6 RB	49.67[a]
10	1:1	6 RB	49.67[a]
2	3:1	1 RB	49.75[a]
5	3:1	1 WB	50.75[a]
6	3:1	6 WB	50.93[a]
1	3:1	—	51.67[ab]
7	3:1	12 WB	53.19[ab]
12	1:1	1 WB	53.57[ab]
9	1:1	1 RB	54.00[ab]
13	1:1	6 WB	54.17[ab]
14	1:1	12 WB	59.14[bc]
8	1:1	—	65.25[c]

Note: Data that share a superscript letter (a, b, c, d) are not significantly different ($P < 0.05$)

on each side). Three days later the room temperature was decreased to 20°C to initiate fruiting. Averaged over all types of substrates, pins were formed 19 days (commercial *P. pulmonarius* strain) to 24 days (*P. pulmonarius* from Nigeria) after inoculation whereas both *P. ostreatus* strains produced pins 23 days after spawning. Statistical analysis was performed on the time/period to complete three flushes on strains (averaged over all types of substrates) and on substrates (averaged over all strains). Significant differences were seen between strains and between substrates in the time needed to complete three flushes (Tables 2 and 3). No significant interaction between strains and substrates was found, indicating that all strains reacted similarly on the different types of substrate. Both *P. pulmonarius* strains had finished three flushes within 45–47 days whereas the *P. ostreatus* strains needed 56–62 days. Due to variation in yield per substrate triplicates, most substrates did

Table 4 Average of yields (fresh weight mushrooms/2 kg bag substrate) and biological efficiency (BE: fresh mushrooms per unit dry weight substrate) for the strains used for three flushes

Strains	*g/2 kg substrate*	*BE (%)*
P. pulmonarius (FIIRO)	331.5[a]	0.5194[a]
P. pulmonarius (commercial)	494.3[b]	0.775[b]
P. ostreatus (Brazil)	505.1[b]	0.7974[b]
P. ostreatus (FIIRO)	583.9[c]	0.915[c]

not differ significantly in the time needed for completion of three flushes. Only the substrate with a 50:50 mixture of peels and stems without supplementation of bran (substrate mixture 8) and supplemented with 12 per cent of wheat bran (substrate mixtures 11 and 14) required significantly longer periods (65 days) for completion of three flushes.

Yields on substrate fermented in trays

Averaged over all substrates, significant differences in yield (fresh mushrooms produced per wet substrate) and biological efficiency (BE, fresh mushrooms per dried unit substrate) were seen between strains for three flushes (Table 4). Both *P. ostreatus* strains produced higher yields than the *P. pulmonarius* strains, although the differences between the highest yielding *P. pulmonarius* and the lowest yielding *P. ostreatus* were not significant. The difference between the lowest (*P. pulmonarius* variety from Nigeria with 319 g of fresh mushrooms per bag of 2 kg) and the highest producer (*P. ostreatus* of Nigeria with 583 g per bag of 2 kg) was *c*. 76 per cent. Averaged over all strains, the total yields after three flushes varied considerably between the different substrates ranging from 347 up to 525 g per 2 kg bag of substrate (Appendix). Previous research has shown that nitrogen content of substrate can improve yields in oyster mushroom cultivation (Dias Nunes et al., 2012). Cassava waste, especially peels, have a low nitrogen content (0.2–0.3 per cent of dry weight; Burns et al., 2012). Other waste streams such as rice and wheat bran have a higher nitrogen content. Rice bran, for example, has a protein content between 10 and 15 per cent which corresponds to a nitrogen content between 1.6 and 2.4 per cent (Fabian and Ju, 2011). Therefore, various amounts of rice or wheat bran were added to the substrate to increase the nitrogen content. The effect of the amount added on mushroom yield differed for the two types of bran. For rice yield the optimum amount is 6 per cent or more whereas for wheat bran the optimum is between 1 and 6 per cent (Figure 2; Table 5). As expected, fruiting bodies of the *P. ostreatus* had a light grey colour whereas those of *P. pulmonarius* were dark brown (Figure 3). It is important to mention that none of the bags was infected (visual inspection for green moulds), despite the fact that the substrates were only fermented for 6 days and maximum temperatures reached varied between 60 and 72.8°C. The individual yields and BE for each strain-substrate combination varied considerably (Appendix) and the *P. ostreatus* variety of Nigeria on substrate mixtures 6, 10, and 12 reached a BE of up to 100 per cent indicating the potential of cassava waste as a substrate for the cultivation of oyster mushrooms (Appendix).

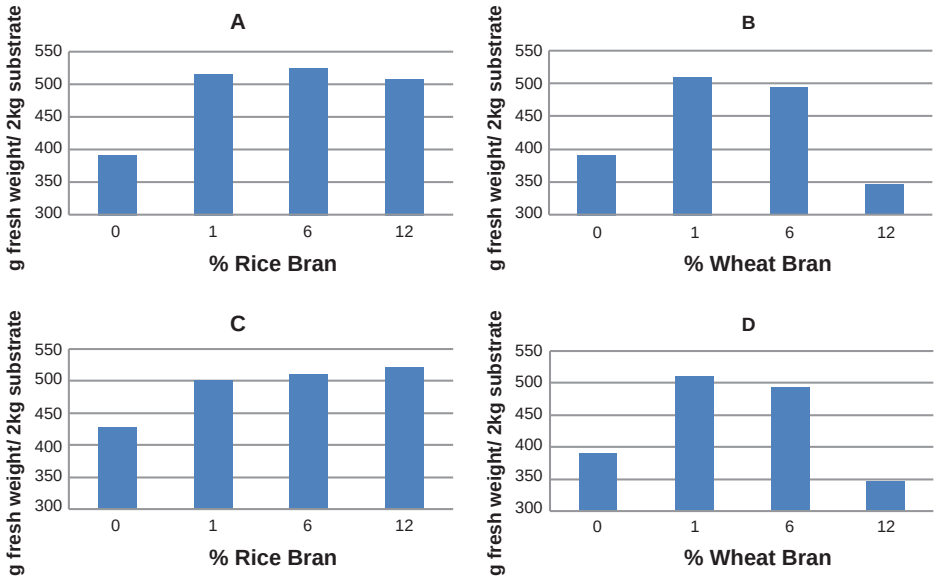

Figure 2 Yield (g fresh weight/2 kg substrate) for increasing supplementation with either rice bran (A and C) or wheat bran (B and D) in substrates with a 1:1 mixture of cassava peels and stems (A and B) or a 3:1 mixture of cassava peels and stems (C and D)

Table 5 Statistical analysis on yields (fresh weight mushrooms/2 kg bag substrate) and biological efficiency (BE: fresh mushrooms per unit dry weight substrate) for three flushes (Tukey's 95% confidence intervals) for each substrate type

Substrate #	*Peels/stems*	*Bran (%)*	*g/2 kg substrate*	*BE (%)*
14	1:1	12 WB	347.2^{a}	59^{a}
8	1:1	—	391.2ab	64^{ab}
1	3:1	—	428.8abc	65^{abc}
7	3:1	12 WB	445.5^{bcd}	73^{bcd}
13	1:1	6 WB	493.8^{cd}	75^{bcd}
5	3:1	1 WB	498.5^{cd}	77^{bcd}
2	3:1	1 RB	501.8^{cd}	77^{bcd}
6	3:1	6 WB	504.5^{cd}	77^{cd}
11	1:1	12 RB	508.1^{cd}	78^{cd}
3	3:1	6 RB	509.7^{cd}	78^{cd}
12	1:1	1 WB	511.5^{cd}	79^{d}
9	1:1	1 RB	515.1^{d}	82^{d}
4	3:1	12 RB	520.8^{d}	83^{d}
10	1:1	6 RB	525.2^{d}	85^{d}

Note: Data that share a superscript letter (a, b, c, d) are not significantly different ($P < 0.05$)

Figure 3 Representative photographs of fruiting bodies of flush 1 of the four varieties grown on substrate prepared from cassava waste

Conclusions

The trials performed with waste prepared from cassava crops from Ghana showed clearly the potential of cassava waste as an ingredient of substrate for the production of oyster mushrooms. Substrates based on cassava waste can give yields of up to 100 per cent BE of oyster mushrooms which compares well to traditional substrates such as sawdust (Frimpong-Manso et al., 2011). Care should be taken not to add too much bran since that will lead to inferior substrates with lower yields. An additional advantage is that substrates based on cassava waste are more quickly colonized than the traditional substrates. Fermentation of cassava waste can be done within 6 days and when done under proper conditions and inoculated under hygienic conditions, sterilization (or heating up to 100°C) is not needed. Cassava-based mixtures thus have the potential to be an economically more profitable production substrate than sawdust-based substrates for the production of oyster mushrooms.

Appendix

Strain	*Substrate*	*Yield average g/2 kg substrate*	*BE average %*
P. ostreatus (Brazil)	14	329.7	59
P. ostreatus (Brazil)	1	395.3	60
P. ostreatus (Brazil)	5	470.3	71
P. ostreatus (Brazil)	8	456.0	75
P. ostreatus (Brazil)	4	510.3	78
P. ostreatus (Brazil)	2	510.7	78
P. ostreatus (Brazil)	11	512.3	79
P. ostreatus (Brazil)	6	531.3	81
P. ostreatus (Brazil)	3	557.0	85
P. ostreatus (Brazil)	12	555.3	90
P. ostreatus (Brazil)	10	573.7	90
P. ostreatus (Brazil)	7	550.0	90
P. ostreatus (Brazil)	9	549.3	90
P. ostreatus (Brazil)	13	569.7	91
P. ostreatus (Nigeria)	14	440.0	74
P. ostreatus (Nigeria)	8	459.0	75
P. ostreatus (Nigeria)	1	561.0	86
P. ostreatus (Nigeria)	7	526.0	86
P. ostreatus (Nigeria)	13	577.3	90
P. ostreatus (Nigeria)	4	599.0	91
P. ostreatus (Nigeria)	2	614.0	94
P. ostreatus (Nigeria)	3	618.0	95
P. ostreatus (Nigeria)	11	617.3	95
P. ostreatus (Nigeria)	5	650.3	98
P. ostreatus (Nigeria)	9	596.0	98
P. ostreatus (Nigeria)	6	648.7	99
P. ostreatus (Nigeria)	12	614.0	99
P. ostreatus (Nigeria)	10	653.7	102
P. pulmonarius (commercial)	14	393.3	66
P. pulmonarius (commercial)	8	416.7	68
P. pulmonarius (commercial)	7	423.3	69
P. pulmonarius (commercial)	1	458.0	70

Strain	*Substrate*	*Yield average g/2 kg substrate*	*BE average %*
P. pulmonarius (commercial)	6	484.0	74
P. pulmonarius (commercial)	11	502.7	78
P. pulmonarius (commercial)	2	514.0	78
P. pulmonarius (commercial)	5	522.0	79
P. pulmonarius (commercial)	10	512.7	80
P. pulmonarius (commercial)	3	540.7	83
P. pulmonarius (commercial)	9	510.3	84
P. pulmonarius (commercial)	13	543.7	84
P. pulmonarius (commercial)	12	524.3	85
P. pulmonarius (commercial)	4	574.7	88
P. pulmonarius (Nigeria)	14	226.0	38
P. pulmonarius (Nigeria)	8	233.3	38
P. pulmonarius (Nigeria)	13	284.3	44
P. pulmonarius (Nigeria)	1	301.0	46
P. pulmonarius (Nigeria)	7	282.7	46
P. pulmonarius (Nigeria)	3	323.0	50
P. pulmonarius (Nigeria)	5	351.3	53
P. pulmonarius (Nigeria)	6	354.0	54
P. pulmonarius (Nigeria)	2	368.7	56
P. pulmonarius (Nigeria)	10	360.7	57
P. pulmonarius (Nigeria)	12	352.3	57
P. pulmonarius (Nigeria)	4	399.0	61
P. pulmonarius (Nigeria)	11	400.0	62
P. pulmonarius (Nigeria)	9	404.7	67

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